

EFFECTS OF CIJI HUA'AI BAOSHENG FORMULA ON APOPTOSIS CORRELATION FACTORS OF TUMOR CHEMOTHERAPY MODEL MOUSE WITH H₂₂ HEPATOMA CARCINOMA CELLS

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Abstract

Background: Side effects of chemotherapy are major issues for cancer patients and there are few methods to release these. However, traditional Chinese medicine is one of the options for these patients. This study is to evaluate one traditional Chinese empirical formula – the Ciji Hua'ai Baosheng Formula (CHBF) on apoptosis related factors in a transplanted tumor chemotherapy model.

Materials and Methods: H₂₂ hepatoma cells were injected into peritoneal cavity and Cytosan (CTX) (200mg/kg) was used to treat these carcinoma cells. Mice were divided into control, CTX control, and CTX plus three different concentrations of CHBF groups. H₂₂ hepatoma cells proliferation, serum levels of apoptosis related factors, and bone marrow cyclin D1 expression was evaluated.

Results: After treatment of CHBF, H₂₂ hepatoma cell proliferation was lower than that in CTX group. The pro-apoptosis related proteins Bax and Caspase-3 were elevated while anti-apoptosis related protein Bcl-2 was reduced. Moreover, serum epidermal growth factor receptor level and bone marrow cyclin D1 expression were significantly reduced in CHBF treated groups.

Conclusion: The Ciji Hua'ai Baosheng Formulae could modulate apoptotic and proliferative factors in a model of tumor with chemotherapy, which may be the mechanisms why it can release chemotherapy related side effect in patients with cancer.

Key words: Ciji Hua'ai Baosheng Formula (CHBF); tumor chemotherapy model; Bax; Bcl-2; Caspase-3; epidermal growth factor receptor (EGFR); CyclinD1; H₂₂ hepatoma carcinoma cell

Introduction

Malignant tumor, a disease of abnormal cell growth, has become more prevalent over the past decade in many countries. In 2012, the incident of cancer in China was 3.065 million, which represented a quarter of global death caused by cancer. Hepatic carcinoma is one of the most common malignant tumors in China, and it is responsible for 43.7% of the world's incidence rate. At the advanced stage of hepatic carcinoma, most patients are treated by chemotherapy, which kills tumor cells but simultaneously destroys normal cells as well. Serious side effects can result during or after treatment that lead to poor prognosis. Traditional Chinese Medicine (TCM), dating back thousands of years, has extensive experience treating hepatic carcinoma based on syndrome differentiation under the concept of holism. By using natural medicinals in individualized prescription, it reduces toxic side effects for postchemotherapy patients, and has gained popularity as an accepted therapeutic method.

The treatment combination of Chinese medical formula is more in line with the principle for cancer therapy, and studies have shown that Chinese herbal medicine can inhibit hepatocellular carcinoma metastasis (Yuan et al., 2009). TCM believes that the pathogenesis of malignant tumor originates from *qi* (energy) stagnation in the body; as a result, blood stasis, phlegm coagulation and toxin gathering soon follow. As liver cancer patients' healthy *qi* declines while pathogenic *qi* gathers, many tumor patients cannot endure the toxic side-effects caused by chemotherapy (Wang, 2004; Wang et al., 2004). Dr. Yanhui Wang, a physician from Medical College of Xiamen University, has concluded based on years of clinical experience in cancer diagnosis and treatment that the toxins and side-effects of chemotherapeutics lie in the disorder of *qi* movement in gallbladder and middle *jiao*. He suggested that treatments should utilize a combination of cold and warm medicinals with methods of eliminating dampness, ascending and descending, astringing and dispersing. And harmonization is the most suitable therapeutics for cancer (Wang, 2004). Dr. Wang stressed the importance in diagnosing cancer through combining tongue and pulse manifestations and laying emphasis on regulating cancer patients' constitution. Based on this belief, Dr. Wang has set up a traditional Chinese empirical formula Ciji Hua'ai Baosheng Formula (CHBF) to treat cancer. The main components include Dangshen (*Radix Codonopsis*), Pingbeimu (Bulbus Fritillariae Ussuriensis), Danshen (*Radix Salviae Miltiorrhizae*), Muli (*Concha Ostreae*), Buguzhi (*Fructus Psoraleae*), Zisuzi (*Fructus perillae Argutae*), Suanzaoren (*Semen Ziziphi Spinosa*), Fuling (*Poria*), Shanzha (*Fructus Crataegi Pinnatifidae*), Chenpi (*Pericarpium Citri Reticulatae*), Houpo (*Cortex Magnoliae Officinalis*), Zhiqiao (*Fructus Aurantii Submaturus*), Chaobaizhu (*Atractylodis Obatae Rhizoma*), Sharen (*Fructus Amomi*), Yizhiren (*Fructus Alpiniae Oxyphyllae*), Baibiandou (*Semen Lablab Album*), which work together to remove pathogenic *qi* and supplement the body.

The constant circulation of *qi* regulates the *yin-yang* balance of the body, and improves the overall internal environment. CHBF has been shown to improve cancer patient survival rate, quality of living, immunologic functions, and reduce postchemotherapy side-effects including mental status decline, hair and appetite loss, insomnia and metabolism dysregulation" (He et al., 2014; Lai et al., 2014). Previous research study confirmed that Ciji Hua'ai Baosheng Granule Formula (CHBGF) prolonged the survival of the mice chemotherapy models of both subcutaneously transplanted tumor and ascitic tumor of H₂₂ hepatoma carcinoma cells. CHBGF exhibited inhibitory effect over the growth of subcutaneously transplanted tumor, and antagonized the decrease in white blood cells and platelets caused by chemotherapy (Xi et

al., 2014).

In order to elucidate the mechanistic action of the formula, the present study focused on investigating the effects of Ciji Hua'ai Baosheng Formula (CHBF) on apoptosis correlation factors using transplanted tumor chemotherapy model mouse with H₂₂ hepatoma carcinoma cells. Research findings aim to offer experimental evidences for its clinical application.

Materials and Methods

Animal and Tumor Cell

A total of 50 specific pathogen-free (SPF) Kunming mice weighing 20±2g, aged 4-6 weeks, male and female in equal numbers were obtained from the SLAC Laboratory Animal Co. Ltd in Shanghai, China [License No. SCXK (Hu) 2012-0002]. H₂₂ hepatoma carcinoma cell suspension was provided by Cancer Research Center of Xiamen University (Xiamen, China) for subcutaneous injection of tumorigenesis.

Experimental Drugs

Ciji Hua'ai Baosheng Formula (CHBF) is composed of Dangshen (*Radix Codonopsis*) 12g (product lot No.130914), Pingbeimu (*Bulbus Fritillariae Ussuriensis*) 30g (product lot No.140130), Danshen (*Radix Salviae Miltiorrhizae*) 50g (product lot No.131217), Muli (*Concha Ostreae*) 50g (product lot No.130828), Buguzhi (*Fructus Psoraleae*) 10g (product lot No.140218), Zisuzi (*Fructus perillae Argutae*) 25g (product lot No.140225), Suanzaoren (*Semen Ziziphi Spinosae*) 25g (product lot No.140130), Fuling (*Poria*) 30g (product lot No.140130), Shanzha (*Fructus Crataegi Pinnatifidae*) 10g (product lot No.140208), Chenpi (*Pericarpium Citri Reticulatae*) 10g (product lot No.140213), Houpo (*Cortex Magnoliae Officinalis*) 5g (product lot No.140303), Zhiqiao (*Fructus Aurantii Submaturus*) 5g (product lot No.140306), Chaobaizhu (*Atractylodis Obatae Rhizoma*) 12g (product lot No.140304), Sharen (*Fructus Amomi*) 5g (product lot No.140303), Yizhiren (*Fructus Alpiniae Oxyphyllae*) 5g (product lot No.140211), Baibiandou (*Semen Lablab Album*) 20g (product lot No.140221), Maiya (*Fructus Hordei Germinatus*) 12g (product lot No.131129), Zelan (*Herba Lycopi Hirti*) 20g (product lot No.140120) and Yimucao (*Herba Leonuri Japonici*) 15g (product lot No.140305), which all were provided by Xiamen Yanlaifu Pharmaceutical Co., Ltd. (Xiamen, China). The combination of all medicinals makes up the equivalent of 351g of crude drug. 0.9% Sodium Chloride Injection, 100 ml each ampoule, product lot No. 1404122311, was produced by Cisen Pharmaceutical Co., Ltd. (Jining, China). CTX for Injection was supplied as a white powder, 0.2g each ampoule, product lot No.131209, was produced by Jilin Tonghua Mao Xiang Medicine Co., Ltd. (Tonghua, China).

Main Reagents

CSB-E08858m Mice Casp-3 ELISA Kit, product lot No. D15014672, CSB-E17114m Mice Bax ELISA Kit, product lot No. D21011789, CSB-E08855m Mice Bcl-2 ELISA Kit, product lot No. G15017949, and CSB-EL007479MO Mice EGFR ELISA Kit, product lot No. D01018741, were produced by Cusabio Biotech Co., Ltd. (Wuhan, China). Primary antibody: CyclinD1 Antibody (H-295), product lot No. Lot#L3217 was produced by Santa Cruz Biotechnology, Inc. (Dallas, USA). Secondary antibody: Anti-Mouse IgG (H+L), product lot No. A5284 was produced by Proteintech Group, Inc. (Chicago, USA). Dehydrated alcohol (China Sanlin Pharma Fujian Co., Ltd.); Haematoxylin staining solution, 0.7% acidized feosin staining solution, and xylene were made by Zhongshan Golden Bridge Biotechnology Co. Ltd, Beijing, China; 10% neutral Formaldehyde Solution, phosphate buffered saline (PBS) and Distilled Water was prepared by the laboratory center of Medical College of Xiamen University, Xiamen, China.

Instruments

Thermo MK3 ELIASA (Thermo Fisher Scientific (China) Co., Ltd., Beijing, China), LeicaRM2016 Histotome (LEICA Co., Solms, Germany), Olympus CX41 Microscope (Olympus Optical Co. Ltd, Tokyo, Japan), TDZ5-WS low-speed multi-pipe carriers autobalance centrifuge (Xiang Yi Centrifuge Instrument Co., Ltd., Changsha, China), ProlinePlus transferpettor (Biohit Co., Ltd., Helsinki, Finland), Counting Slide for Counting Blood Cells (Shanghai Qiuqing Biochemical Reagent & Instrument Co., Shanghai, China) and GNP-9270 incubator (Shanghai Jing Hong Laboratory Instrument Co., Ltd., Shanghai, China)

Modeling and Mice Breeding

The mice were bred in an animal house (SPF degree) with barrier system assisted with apinoid laminar flow chamber in the Experimental Animal Center of Xiamen University, and used for experiment after 1 week. Hydroperitoneum mice with H₂₂ hepatoma carcinoma cells were transferred until ivory white hydroperitoneum could be suctioned. Hydroperitoneum suctioning was done under aseptic conditions, and placed on a counting slide under an inverted microscope (×100). The concentration was regulated with normal saline, and the single cell suspension with 2×10⁷ cells/ml was counted. 0.2ml suspension (about 4×10⁶ cells) was inoculated subcutaneously to the right armpit or peritoneal cavity under aseptic condition. Seven days after inoculation, 50 mice all formed the transplanted tumors, were used for peritoneal injection of Cytosan (CTX) at the dosage of 200mg/kg to establish the chemotherapy model with subcutaneously transplanted tumor of H₂₂ hepatoma carcinoma cells.

Drugs Preparation

All herbal medicinal pieces of CHBF were soaked in appropriate amounts of distilled water (1:8) for 20min, decocted for 30min, and

filtered with 8 layers of carbasus. Then, the gruffs were decocted in water (1:6) for 30min, and filtered with 8 layers of carbasus. The two solutions were combined, evaporated and concentrated to 5.85g/ml, 2.93g/ml and 1.46g/ml respectively. They were preserved in fridge at 4°C. CTX was made by dissolving crude power with 0.9% sodium chloride solution to a final concentration of 1.66mg/ml.

Animal Grouping and Administering

Fifty mice with tumors were used for peritoneal injection of Cytoxan (CTX) (200mg/kg) to establish the chemotherapy model mouse on Day 0. They were randomly divided into 5 groups of 10 mice: model, CHBF with high dosage (CHBF-H, 117g/kg), CHBF with middle dosage (CHBF-M, 58.5g/kg), CHBF with low dosage (CHBF-L, 29.25g/kg) and a positive control (CTX, 33.33mg/kg). On Day 1, medicines were administered intragastrically to each corresponding group: normal saline (0.9%, 0.2ml/10g), CHBF-H (5.85g/ml, 0.2ml/10g), CHBF-M (2.93g/ml, 0.2ml/10g, equivalent to 20 times the human dosage) and CHBF-L (1.46g/ml, 0.2ml/10g) once a day. Positive control was treated with peritoneal injection of CTX (1.66mg/ml, 0.2ml/10g) once every other day. Mice were treated for the next 10 days.

Content of Serum Bax, Bcl-2, Casp-3 and EGFR Detected by ELISA

Twenty-four hours after the last administration, 0.8mL of peripheral blood was collected from each mouse through eyeball extirpation. Blood samples were stored at room temperature for 2 hours before retrieving the serum by centrifuging for 15min with 3000r/min at 4°C. 100µl of each sample was incubated in primary and secondary antibodies, and subsequently the optical densities (OD) were measured by ELISA in 450 nm wavelength.

Preparation of Pathological Section and Observation

Tumor tissues were collected after mouse sacrifice, and fixed in formaldehyde solution for 12 hours. Samples were dehydrated in sequential steps and embedded in paraffin. Paraffin tissue sections were prepared at 5µm thickness on a glass slide and stained with hematoxylin and eosin. The tumor pathology was viewed under 400× magnifications for oncopathological changes.

Protein Expression of CyclinD1 in Left Femur Bone Marrow Detected by Immunohistochemistry

According to the above-mentioned procedures, tissue sections were dewaxed with xylene and washed in decreasing concentrations of alcohol. They were then treated with 10% blood serum and incubate in incubator at 37 °C for 30 min before CyclinD1 primary antibody was added. PBS was used for control tissue. Samples were preserved at 4 °C overnight, and secondary anti-mice IgG antibody was added the next day. After immunostaining, tissues were counterstained with hematoxylin and viewed under microscopy. The brown yellow dots in cell nucleus and cytoplasm represented positive expression of CyclinD1 by immunohistochemistry. Five field views under 40× objective lens, were randomly selected on each tissue section using Image-Pro Plus image analysis Software. The integrated optical density value (IOD) was measured, and an average value of the five views was calculated. The IOD value represented the expression intensity of CyclinD1.

Statistics

The experimental results were expressed as mean ± standard deviation ($\bar{x} \pm s$). Statistical Product and Service Solutions (SPSS) 19.0 (IBM, Armonk, NY, USA) was used for one-way analysis of variance [One-Way ANOVA (analysis of variance)]. The least significant different (LSD) method was chosen. $P < 0.05$ was regarded as statistically significant different.

Results

Effects of CHBF on Pathological Change of Transplanted Tumor Chemotherapy Model Mouse

Most tumor masses had clear border formation. The tumor tissue was firm and light yellow in color, the shape was ellipsoid, and the cross-sectional area was pale in color. The tumor tissue in model group was irregular and had a rough surface. After the tissue section was stained, as indicated in Figure 1 under the inverted microscope, H₂₂ hepatoma carcinoma cells in the model group were over populated with cells undergoing cell divisions. H&E staining revealed markedly prominent nuclei and irregular cell organization. Tumor cells in the CTX group were scarce and scattered. After intervention of Chinese medical formula, tumor cells in the three CHBF manifested a lower distribution density of tumour cells than that of model group, and their disordered arrangement was also not more than that of model group, which may be related to the dosage of CHBF.

Effects of CHBF on Content of Serum Bax, Bcl-2, Casp-3 and EGFR of Transplanted Tumor Chemotherapy Model Mouse

As indicated in Table 1, treatment with CHBF increased the expression of serum Bax and Caspase-3 proteins, while decreased the levels of Bcl-2 and EGFR proteins. Bax protein in CHBF-M group was significant higher as compared to the CTX group ($P < 0.05$) and model group ($P < 0.01$), while CHBF-L group showed greater statistical significant difference as compared to both CTX and model groups ($P < 0.01$). Bcl-2 protein content in all CHBF groups was significant decreased as compared to both CTX and model groups ($P < 0.01$) with the most significant reduction in CHBF-H group. The Caspase-3 protein content in CHBF-L and CHBF-M groups increased significantly as compared

to the model and CTX groups ($P<0.01$), while CHBF-H group was moderately increased when compared to the model and CTX groups ($P<0.05$). Compared with the model group, content of EGFR protein in CHBF-L, CHBF-M ($P<0.05$) and CHBF-H ($P<0.01$) groups were significantly lower. However in comparison to the CTX group, only CHBF-H group showed significant difference ($P<0.05$).

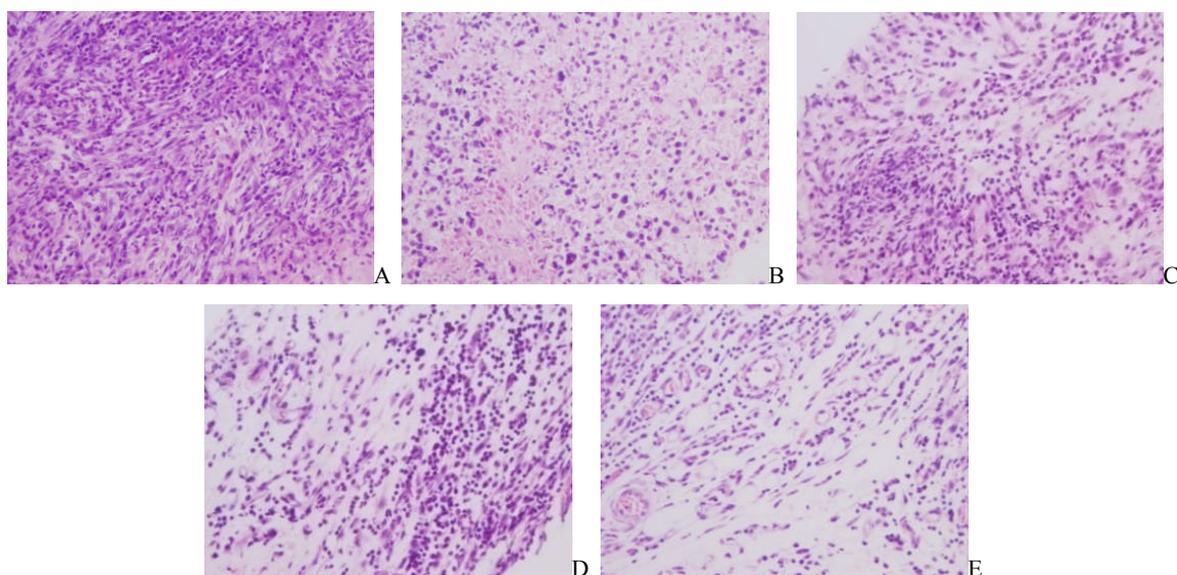


Figure 1: Pathologic diagram of the transplanted tumor chemotherapy model mouse with H₂₂ hepatoma carcinoma cells in each group (400×, H&E staining). A: Model group; B: CTX group; C: CHBF-H group; D: CHBF-M group; E: CHBF-L group.

Table 1: Effect of CHBF on serum Bax, Bcl-2, Caspase-3 and EGFR protein content of H₂₂ transplanted tumor chemotherapy model mouse ($\bar{x} \pm s$)

Group	Dose(g/kg)	n	Bax (pg/ml)	Bcl-2 (pg/ml)	Caspase-3 (ng/ml)	EGFR (ng/ml)
Model	--	8	1132.48±375.53	1845.60±563.47	0.74±0.24	179.68±35.28
CTX	0.033	9	1356.17±302.07	1718.99±500.03	0.77±0.29	164.40±16.43
CHBF-H	117	7	1450.94±317.51	534.86±218.11 ^{bd}	1.03±0.22 ^{ac}	130.25±28.79 ^{bc}
CHBF-M	58.5	10	1739.20±393.52 ^{bc}	879.49±189.55 ^{bd}	1.09±0.18 ^{bd}	144.41±27.14 ^a
CHBF-L	29.25	10	1923.85±282.80 ^{bd}	894.76±339.00 ^{bd}	1.30±0.29 ^{bd}	147.96±38.44 ^a

Notes: ^a $P<0.05$, ^b $P<0.01$ vs. the model group; ^c $P<0.05$, ^d $P<0.01$ vs. the CTX group

Effects of CHBF on Protein Expression of CyclinD1 in Left Femur Bone Marrow of Transplanted Tumor Chemotherapy Model Mouse

As indicated in Figure 2, there was a high expression level of CyclinD1 protein in model group as shown by brownish yellow marks distributed extensively through the cytoplasm. CyclinD1 expression in CTX group was slightly less as compared to the model group. The expression of CyclinD1 protein in three CHBF groups was greatly decreased. As indicated in Table 2 and Figure 3, the CyclinD1 protein expression in left femur bone marrow in CHBF-H (117g/kg) group was significantly decreased as compared to the model and CTX groups ($P<0.01$); this trend was also observed in CHBF-M (58.5g/kg) group as compared to the model group ($P<0.05$).

Discussion

The pathogenesis of malignant tumor has been the research focus for decades due to difficulties in treatment. Apoptosis is the process of autonomic cell death that is strictly regulated by many genetic factors. It is closely related to carcinogenesis and metastasis, and plays a pivotal role in treatment, transformation and prognosis of cancer (Burikhanov et al., 2013; Feng et al., 2012). Some researches utilize cell apoptosis as a marker to evaluate the therapeutic effect of malignant tumor and attribute tumor occurrence to insufficiency of tumor cell apoptosis (Jana et al., 2007; Meiler et al., 2006). Bcl-2 and Bax are genes related with apoptosis; the former is anti-apoptosis while the latter is pro-apoptotic (Liang et al., 2012; Yeo et al., 2012). Previous research discovered that Bcl-2 is overexpressed in tumor diseases (Adams et al., 2007). As a result, Bcl-2 proteins dimerize, which promote the formation of Bcl-2/Bax heterodimers; and both complexes can decrease apoptosis. When Bax is expressed and formed homodimer, it will promote apoptosis (Chen et al., 2014). There are 14 types of cysteine-containing aspartate-specific proteases (Caspase) found in the human body. They are categorized according to their functions in apoptosis: caspase-3, caspase-6, caspase-7 are initiator caspases, and caspase-2, caspase-8, caspase-9 and caspase-10 are effector caspases. Under the induction of apoptotic signal, initiator caspases activate pro-effector caspases, and together facilitate the apoptotic progress (Blanc et al., 2000). Among the caspases, caspase-3 is the most important effector enzyme during apoptosis (Blanc et al., 2000). EGFR (epidermal

growth factor receptor) belongs to the tyrosine kinase receptor family and plays an important role in the development of malignant tumor. Studies indicated that many solid tumors have abnormally high expression of EGFR and is related to tumor cell proliferation, neoplasm invasiveness and apoptotic inhibition (Lui et al., 2002; Liu et al., 2003). Modification of either the ligand binding region or the tyrosine kinase region of the EGFR influenced the downstream signaling pathway, and is associated with the occurrence of malignant tumor of many types such as breast cancer, esophageal cancer, colon carcinoma, lung cancer and cervical cancer (Raymond et al., 2000). The expression level of EGFR in hepatic carcinoma is positively correlated with its malignancy. One proposed mechanism may be the increase of EGFR ligands persistently activates EGFR, which upregulates downstream signal transductions and downregulates EGFR receptor recycling (Zhao et al., 2004; Tidow et al., 2003; McKay et al., 2002). During the cell cycle, progression from G phase to S phase is a key point in which extracellular and intracellular signals gather at the nucleus and regulate cell proliferation (Viallard et al., 2001). CyclinD1 protein is an important regulator of cell cycle that specifically binds cyclin dependent kinase (CDK) to promote cell conversion from G phase to S phase; overexpression of the protein leads to unrestrained cell proliferation and carcinomatous changes (Li et al., 2010). Thus, regulation of EGFR and CyclinD1 is vital in inhibition of tumorigenesis, and plays a synergetic role in promoting apoptosis.

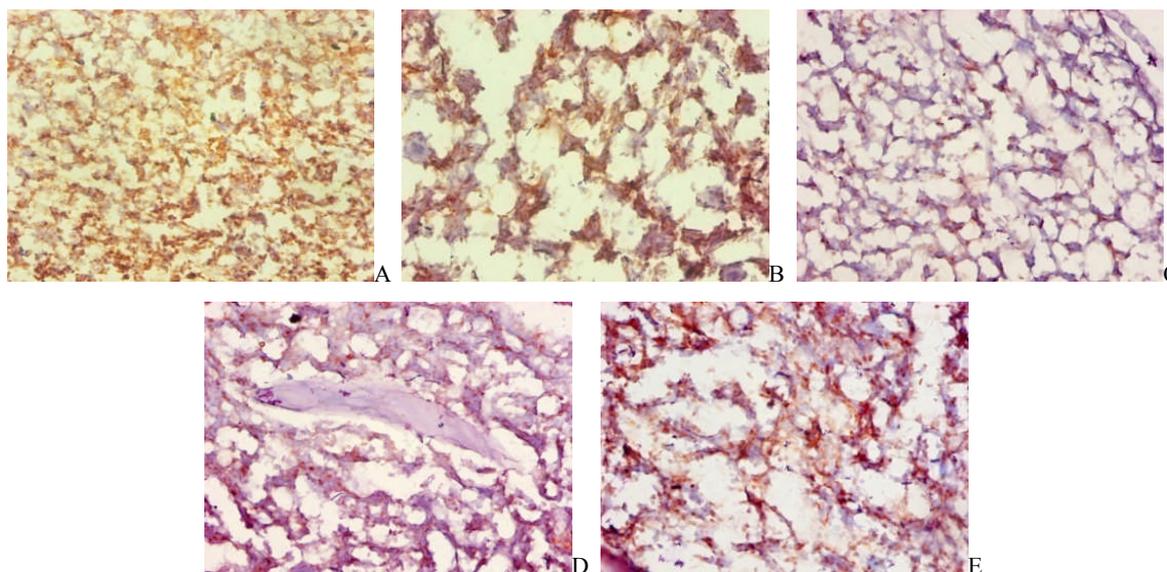


Figure 2: Immunohistochemistry image of CyclinD1 protein expression in left femur bone marrow of the transplanted tumor chemotherapy model mouse with H₂₂ hepatoma carcinoma cells in each group (400×, IHC staining). A: Model group; B: CTX group; C: CHBF-H group; D: CHBF-M group; E: CHBF-L group.

Table 2: Effect of CHBF on average IOD of CyclinD1 protein expression in left femur bone marrow of H₂₂ transplanted tumor chemotherapy model mouse ($\bar{x} \pm s$)

Group	Dose(g/kg)	n	IOD
Model	--	7	35674.10±6447.53
CTX	0.033	7	34813.59±5525.07
CHBF-H	117	7	22776.85±9060.61 ^{bd}
CHBF-M	58.5	7	30923.29±9822.16 ^a
CHBF-L	29.25	7	32687.72±10554.78

Notes: ^a P<0.05, ^b P<0.01 vs. the model group; ^c P<0.05, ^d P<0.01 vs. the CTX group

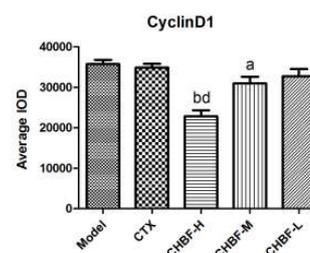


Figure 3: Effect of CHBF on average IOD of CyclinD1 protein expression in left femur bone marrow of H₂₂ transplanted tumor chemotherapy model mouse in different groups. LSD Statistical analysis: ^a P<0.05, ^b P<0.01 vs. the model group; ^c P<0.05, ^d P<0.01 vs. the CTX group. n= 7 animals/group.

With the rapid development of modern pharmacology, multiple effects of Chinese herbal medicines and traditional Chinese empirical formulas on tumor cell apoptosis have been brought to light. Study has revealed that the volatile oil of *Semen Sinapis* can inhibit tumor cell growth in H₂₂ tumor-bearing mice through mechanisms that upregulate Bax and downregulate Bcl-2 expressions to induce apoptosis (Nan et al., 2014). The extract of *Herba Scutellariae Barbatae* was also shown to inhibit proliferation and induce apoptosis of hepatoma H₂₂ cells via the mitochondrial pathway involving caspase-3 (Dai et al., 2008). Furthermore, Peony Liver-Softening Formula demonstrated anti-tumor action by down-regulating the mRNA expression of CyclinD1 in tumor-bearing mice, and inhibited the proliferation of H₂₂ and S180 tumor cells (Xu et al., 2011).

Our experimental model mice treated with Ciji Hua'ai Baosheng Formula after chemotherapy closely resemble treatment state of clinical patients. In CHBF, Dangshen (*Radix Codonopsis*), Chaobaizhu (*Atractylodis Obatae Rhizoma*), Fuling (*Poria*), Shanzha (*Fructus Crataegi Pinnatifidae*), Maiya (*Fructus Hordei Germinatus*), Chenpi (*Pericarpium Citri Reticulatae*) and Baibiandou (*Semen Lablab Album*) used after tumor chemotherapy can boost *qi* and supplement the spleen; Buguzhi (*Fructus Psoraleae*) and Yizhiren (*Fructus Alpiniae*)

Oxyphyllae) work to warm the spleen and kidney; Pingbeimu (Bulbus Fritillariae Ussuriensis), Zisuzi (*Fructus perillae Argutae*), Houpo (*Cortex Magnoliae Officinalis*), Zhiqiao (*Fructus Aurantii Submaturus*) and Sharen (*Fructus Amomi*) can rectify *qi* and dissolve phlegm; Danshen (*Radix Salviae Miltiorrhizae*), Yimucao (*Herba Leonuri Japonici*), Zelan (*Herba Lycopi Hirti*) and Muli (*Concha Ostreae*) can dissolve stasis and dissipate masses; Suanzaoren (*Semen Ziziphi Spinosae*) and Muli (*Concha Ostreae*) can calm the mind. The formula lays emphasis on balance of diet, sleep and metabolic functions in cancer patients, which has effects on both dispelling pathogens and reinforcing healthy *qi*. Treatments with CHBF accentuate on returning health *qi* back to the whole body system, which is the essence of TCM for cancer postchemotherapy patients.

The aim of the present study is to determine whether CHBF had an effect on promoting apoptosis and inhibiting proliferation of tumor cells in postchemotherapy mouse. Our findings indicated that CHBF in different dosages could ameliorate tumor pathological changes, lower tumor cell growth and distribution, and inhibit tumor cell proliferation. CHBF increased the content of serum pro-apoptotic genes Bax and Caspase-3, especially in CHBF-M (58.5g/kg) and CHBF-L (29.25g/kg) groups. Moreover, CHBF decreased the content of apoptosis inhibitory gene, Bcl-2, cell proliferation regulation correlated gene, EGFR, and the expression of femur pro-proliferative gene CyclinD1 protein. In these cases, CHBF at the highest dosage of 117g/kg had a better inhibitory effect. Interestingly, treatment with CHBF at any concentration was more effective at reducing tumor progression than the use of chemotherapy drug CTX. These results provided scientific evidence for the clinical application of Ciji Hua'ai Baosheng Formula. In order to elucidate the mechanisms behind CHBF promoting cell apoptosis and inhibiting tumor proliferation, future research should focus on individual active constituents of CHBF that reflect its therapeutic effect.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No.81202659), the Xiamen Science and Technology Key Program Plan Grant (No.3502Z20100006), the Natural Science Foundation of Fujian (China) (No.2014J01373) and the Scientific Research Start Foundation for New Teacher of China Xiamen University (ZK1014). We gratefully thank the experimentalist Fu Chen of the Medicine Laboratory Animals Center of Xiamen University for assisting in laboratory procedures.

Declaration of Interest

All the authors declare no actual or potential conflicts of interest. All authors are responsible for the content and writing of the paper and approved of its publication.

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